Prevalence and Antimicrobial Susceptibility Pattern of Uropathogenic Escherichia coli among Adult Diabetic Patients Attending Federal Medical Centre Gombe, Gombe State Nigeria

By

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ABSTRACT

Two hundred urine samples were collected from patients attending diabetic clinic at the Federal Medical Centre Gombe (FMCG). The samples were cultured on nutrient agar and the isolates obtained were Gram-stained, subjected to biochemical tests to confirm their identities. All the 200 samples were found to contain Gram negative organisms. Of the 200 gram negative isolates obtained, 111(55.5%) were found to be positive for *E. coli* while the remaining, 89(44.5%) were found to be negative for *E. coli* but positive for other enteric gram negative rods(Enterobacteriaceae). Female diabetic patients recorded higher incidence of 85(77%) than their male counterparts of 26(23%). Antimicrobial susceptibility testing was carried out against the isolated *E. coli* using Kirby-Bauer (1966) method and shows the activity of only members of Aminoglycosides, streptomycin and Gentamycin as moderately sensitive and highly sensitive respectively. Other antibiotics proved inactive, to which the *E. coli* became resistant, and these include Septrin, chloramphenicol, quinolones (Sparfloxacin, ciprofloxacin, Peplofaxcin and Tarivid) and augmentin (Maxi high profile Gram negative antibiotics).

Keywords: Uropathogenic, *Escherichia coli*, antimicrobial, susceptibility, antibiotics, diabetic.

INTRODUCTION

*Escherichia coli* are Gram negative, facultatively anaerobic, non-sporulating motile rods. Inactive strains are non-motile and a few of the strain are capsulated. *E. coli* is a bacterium found in the lower intestines of warm-blooded animals. Most *E. coli* strains are harmless, but some such as serotype O157: H7 can cause serious food poisoning. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections (UTI), wound infections, peritonitis, sepsis and endotoxin-induced shock. *E. coli* is motile, urease negative, indole positive, lactose fermenter, does not produce hydrogen sulphide, gas producer on KIA with yellow butt and yellow slope (Cheesbrough 2000, Geo et al., 2001,).

*E. coli* is the most common causative agent of urinary tract infections in both diabetes mellitus (DM) and non-diabetes mellitus patients. Other organisms reported include members of the family Enterobacteriaceae (Proteus, Klebsiella, Enterobacter and Citrobacter species), Pseudomonas species, Enterococcus species, Streptococci, Staphylococci and *Candida albicans* (Ludwig,2000).

Uropathogenic *E. coli* (UPEC), a bacteriuria-causing *E. coli* possesses a variety of pathogenicity determinants that make colonization of the urinary tract possible. These include fimbral (PS/F1C and type 1 fimbiae) and non-fimbrial adhesins that mediate bacterial adherence to epithelial cells, siderophores (iron-acquisition systems), secreted toxins (haemolysin and cytotoxic necrotizing factor 1) and capsule forming polysaccharides for immune evasion (Bower et al.2005; Tseng et al., 2003).

Phylogenetic analysis classifies *E. coli* strains into four main groups (A, B1, B2 and D). Group B2 and D are mainly associated with *E. coli* strains causing extraintestinal infections, whilst group A and B1 are associated with
commensal strains (Picard et al., 1999). *E. coli* is the most common cause of UTI and accounts for approximately 90% of first UTIs in young women (Geo et al., 2001).

The disease Diabetes Mellitus is known to mankind from time unknown. In India, the disease was described by Charaka Sanhita (2nd century A.D.). In Egypt (Around 1500 B.C.), the melody of polyuria was mentioned in medical complication by the Egyptian Payrus of Ebers (Khalipha 2011).

Diabetes Mellitus (DM) was reported to be the fifth largest death causing disease in United States in 1974. Prevalence of the disease has been increasing, being doubled between 1965 and 1973. A 6% increase per year has been observed thereafter. As per national diabetic data, 11.2% of the population has been found to be impaired of glucose tolerance. The first authentic data of the prevalence of 2.3% in urban and 1.5% in rural set ups. The incidence is seen to be parallel with social and cultural changes including increased stress and dietary habits (Khalipha 2011).

Diabetes affects many systems that protect against infection in general, and against urinary tract infections, specifically. In UTIs, women with diabetes are more likely to have bacteria in their bladders than women without diabetes, though the same does not appear to be true in case of men. There also seems to be an increased risk of infection spreading upwards into the kidneys of diabetic patients, and diabetic women with UTIs are more likely to acquire hospitalization (Kalipha 2011).

Urinary tract infection is one of the most common bacterial infections in women, and it is estimated that as many as 60% of all women report having had a UTI at least once in their lifetime (Sita et al., 2006). *E coli* are the most frequently isolated urinary pathogens, which accounts for 5 to 90% of all uncomplicated urinary tract infections. It is now recognized that there are subsets of faecal *E. coli*, which can colonize periurethral area, enter urinary tract and cause symptomatic disease. These are currently defined as Uropathogenic *E. coli* (UPEC). These isolates express chromosomally encoded virulence markers (Raksha et al., 2003).

This study was conducted with the objective of finding the incidence of Uropathogenic *E. coli* among diabetic patients attending Federal Medical Centre Gombe, and their possible treatments using antimicrobial susceptibility testing.

**MATERIALS AND METHODS**

**Sample collection site**

The site for collecting the sample was a diabetic clinic at the federal medical centre Gombe with the aid and consent of Medical Research Ethic Committee (MREC) through the office of the director administration, FMCG. This justifies the choice for the diabetic clinic as the study site as, patients with diabetes from within and outside the state patronize the clinic for the treatment of diabetes and its concomitant complications especially bacterial infections, the major of which is bacteriuria caused by the uropathogenic *E. coli* (UPEC) (Geerling et al., 2000, Shah et al., 2003, Muller et al., 2005).

**Culturing of Samples**

Two hundred mid-stream urine samples were collected in sterile sampling bottles from the diabetic clinic at FMCG in Gombe, north eastern Nigeria and quickly transported to the Microbiology laboratory, Gombe state university for analysis. The samples were inoculated onto nutrient agar plates, gram-stained and sub-cultured on Eosin Methylene Blue (EMB) agar and McConkey agar for necessary identification of Enterobacteriaceae and lactose fermentation respectively (Cheesbrough 2000).

**Gram’s staining reaction**

The isolates obtained from the nutrient agar plates were subjected to Gram’s staining for separation into gram positive and gram negative using standard procedure (Cheesbrough 2000, Geo et al., 2007). This is because the organism in question is a gram negative organism.

**Identification of the gram negative isolates**

The gram negative isolates obtained were cultured on EMB agar and McConkey agar for their identities as members of Enterobacteriaceae and lactose fermenters respectively. Further biochemical tests which include urease test, citrase test, motility test, ornithine decarboxylase test, indole production test as well as gas and hydrogen sulphide
production test using Kligler iron agar (KIA) were also carried out to confirm their identities using standard procedure (Cheesbrough, 2000).

Standardization of inoculum

Following the confirmatory tests for the organism and sub culture of the confirmed *E. coli* isolates on nutrient broth for 24 hours at 37°C, loopfuls of the isolates were emulsified in normal saline to match the turbidity standard of 0.5 McFarland (Cheesbrough, 2000) for antimicrobial susceptibility testing. This is according to suggestion made by the National Committee for Clinical Laboratory Standard (NCCLS, 1999).

Antimicrobial Susceptibility testing

The prepared suspension of the standard inoculum was swabbed on to the surface of Mueller Hinton agar plate and the commonly used standard antibiotic sensitivity discs were aseptically placed on top of the inoculated plates. The Petri plates were later kept in an incubator at 37°C for 24 hours in an inverted position for proper diffusion of the antimicrobials.

RESULTS

Table 1: Prevalence of *E. coli* isolated from urine sample the diabetic patients.

<table>
<thead>
<tr>
<th>Total urine samples collected</th>
<th>Samples positive for <em>E. coli</em></th>
<th>Samples negative for <em>E. coli</em> but positive for other Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>111 (55.5%)</td>
<td>89 (44.5%)</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of *E. coli* isolated from urine samples of diabetic patients based on gender.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Samples collected</th>
<th>Number positive (%)</th>
<th>Number negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>65</td>
<td>26 (40%)</td>
<td>39 (60%)</td>
</tr>
<tr>
<td>Female</td>
<td>135</td>
<td>85 (63%)</td>
<td>50 (37%)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>111 (55.5%)</td>
<td>(44.5%)</td>
</tr>
</tbody>
</table>

Table 3: Antimicrobial susceptibility testing of *E. coli*.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Antibiotics</th>
<th>Concentration(µg/disc)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Septrin (30)</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol (30)</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Sparfloxacin (30)</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin (30)</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin (30)</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Augmentin (30)</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Gentamycin (10)</td>
<td></td>
<td>32.00</td>
</tr>
<tr>
<td></td>
<td>Pefloxacin (30)</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Tarivid (30)</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Streptomycin (10)</td>
<td></td>
<td>21.00</td>
</tr>
</tbody>
</table>

DISCUSSION

Table 1 shows the incidence of *E. coli* isolated from the urine samples of 200 patients, out of which 111 samples tested positive for *E. coli* while 89 were negative, but positive for other enteric gram negative rods. This shows that *E. coli*, not only in diabetic patients, is the most common cause of urinary tract infections accounting for about 90% occurrence (Geo et al., 2001, and Raksha et al., 2003). Bacteriuria is therefore more frequently to be caused by *E. coli*.
Male diabetic patients are less likely to suffer *E. coli* infections as, out of a total of 65 urine samples collected from male diabetic patients only 26(40%) were found to be positive for *E. coli* and the remaining 39(60%) proved negative(Table 2).

However, 135 urine samples were collected from female diabetics, out of which 85(63%) tested positive for *E. coli* as against the 50(37%) negative ones (Table 2). This work agrees with that of Patterson et al., who found high level of infection in the urinary tract of diabetic women which may be determined by the number of microorganisms located in the vagina. *E. coli* was the most commonly recovered uropathogen in diabetes mellitus patients with urinary tract infections in this study (Hansen et al., 1998, Lye et al., 1992; Geo et al., 2001).

Although our findings are similar as far as *E. coli* is concerned, to those of Bonadio et al., (1999), the differences in the investigation of other uropathogens might not be unconnected with the different populations studied.

Table 3 shows antimicrobial susceptibility testing of high profile gram negative antibiotics (Maxicare) on the isolated *E. coli* isolates. Septrin, Chloramphenicol, members of the quinolones (Sparfloxacin, Ciprofloxacin, Pefloxacin and Tarivid), Amoxycillin and Augmentin all proved inactive against the *E. coli* isolated while only the two members of the Aminoglycosides, Gentamycin and Streptomycin were found to be active with the latter being highly sensitive and the later moderately sensitive.

This study therefore corresponds with the work of Bonadio et al., (2001), who found out that antimicrobial resistance is increasing among uropathogens (*E. coli* as the major) causing community and nosocomial UTIs. But for diabetes mellitus as a risk factor for the development of uropathogens antimicrobial resistance, few data are still available. Recent study in Italy reported that the resistance of uropathogens to antibiotics was similar in patients with and without diabetes mellitus (Bonadio et al., 2001).

However, all classes of uropathogens as reported by previous findings, regardless of their source, showed high rates multiple antimicrobial resistance, particularly to some of the drugs commonly used for the treatment of UTIs, and almost all the *E. coli* isolated in this study were resistant to quinolones and other aforementioned drugs tested (Bonadio et al., 2001).

**CONCLUSION**

Female diabetic patients are more prone to the infections caused by the Uropathogenic *E. coli*, than men and there is an increased resistance of *E. coli* to some quinolones, amoxicillin, augmentin, chloramphenicol and Septrin and high sensitivity to Gentamycin with moderate sensitivity to streptomycin.

**RECOMMENDATION**

Further research should be carried out to find out if at all diabetes mellitus contributes in the development of antimicrobial resistance of Uropathogenic *E. coli* and other uropathogens.

**ACKNOWLEDGEMENT**

We wish to acknowledge the effort of the diabetic clinic of the Federal Medical Centre Gombe (FMCG) as well as that of the Medical Research Ethic Committee (MREC) of the said FMC for their endurance and dedication of giving us the needed samples alongside their needed information.

**REFERENCES**


